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RESEARCH ARTICLE

RSM-optimization of microwave-assisted extraction of *R. laevigata* polysaccharides with bioactivities

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ABSTRACT

Rosa laevigata Michx. is an edible traditional Chinese medicinal herb that possesses several health benefits. This study optimized the microwave-assisted extraction conditions of polysaccharides from *Rosa laevigata* fruits and investigated the antimicrobial potency and the antioxidant activity of *Rosa laevigata* polysaccharides. The microwave power, extraction time, the number of extraction, and the solvent-to-solid ratio were established using a three-factor, three-level Box-Behnken Design (BBD) and Response Surface Methodology (RSM) using screening experiments. Maximum polysaccharides yields were obtained when extraction was done for 18 min at a microwave power of 516 W and using a liquid-solid ratio of 54 mL/g. The polysaccharides yield was 20.37 % under the optimized conditions (predictive value for 20.40 %). This study also determined the antioxidant activity and the antibacterial potency of *Rosa laevigata* fruits to enhance their functional characterization and medicinal use. The antioxidant experiment showed that when the concentration was 5.0 mg/mL, polysaccharides had the strongest scavenging ability to DPPH free radicals and ABTS free radicals, and the scavenging rate was 83.06 % and 99.38 % respectively. Furthermore, the antibacterial experiment showed that the polysaccharides exhibited prominent inhibition activities, the MIC against *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* at 3.13 mg/mL and 1.56 mg/mL, respectively.

Keywords: *Rosa laevigata* Michx.; Polysaccharides; Microwave-assisted extraction; Antibacterial activity; Antioxidant activity

INTRODUCTION

Rosa laevigata Michx. (Rosaceae family) is a popular edible plant with pharmacological potency. The fruits of *Rosa laevigata* have been employed for the cure of chronic cough, frequent urination, and arterial sclerosis (Liu et al., 2013). *Rosa laevigata* fruits are composed of polysaccharides, flavonoids, saponins, organic acids and tannins among other compounds (Dong et al., 2015; Yan et al., 2011). The polysaccharides in *R. laevigata* possess antioxidant, hepatoprotective, antibacterial, and anti-inflammatory activities (Liu et al., 2018; Yu et al., 2013). There is, therefore, a growing research interest on the polysaccharides of *R. laevigata* fruits.

Extraction methods affect the yield, chemical structures and the biological activities of medicinal plant extracts. Microwave-assisted extraction provides an avenue for fast and efficient isolation of bioactive compounds from various plants (Bhan et al., 2017). Such as, extraction of curcumin from turmeric, (Doldolova et al., 2021) total saponins from the starfish *Echinaster sepositus* (Dahmoune et al. 2021) and proanthocyanidins from *Cinnamomum camphora* leaves (Liu et al. 2021) using microwave assisted method. The microwave is a type of electromagnetic radiation with a frequency between 300 MHz and 300 GHz. During the extraction process, the microwave instrument generates an electromagnetic field, which raises the temperature and causes the cells to burst. Subsequently, the compounds within the cells are released into the solvent resulting in rapid and efficient extraction of compounds (Gabriel et al., 1998). Microwave-assisted extraction offers several advantages over traditional extraction methods. These

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advantages include a significant reduction in extraction time, reduction in the amount of organic solvent, reduced energy consumption and improved the yield (Bhan et al., 2017; Gabriel et al., 1998; Zhao et al., 2016). The Response Surface Method (RSM) is a popular statistical method used to optimize experimental conditions and helps to reduce the number of preliminary tests required to evaluate multiple parameters and their interactions (Filip et al., 2017). The RSM was first introduced in 1951 by Box and Wilson for the optimization of chemicals extracted from different plants. The RSM can be effectively applied to evaluate the effects of multiple factors and their interactions on one or more responses. Also, the RSM can precisely define the interaction between the various extraction factors from the model (Azahar et al., 2017). This study used RSM to optimize the microwave extraction method for polysaccharides from *R. laevigata* fruits.

Our previous studies indicated that the polysaccharides of *R. laevigata* fruits possess neuroprotective and antioxidant profiles (Liu et al., 2018). The increasing interest in the medicinal potency of this plant prompted us to explore its other potential values. It has been reported that the extraction rate of plant polysaccharides by water extraction and alcohol precipitation is low (Luo et al., 2014; Zhao et al., 2016). The microwave-assisted method not only greatly shortens the extraction time but also relatively upgrades the yields (Lu et al., 2019). This study aimed to optimize the microwave-assisted extraction process of polysaccharides from *R. laevigata* fruits by RSM (Azahar et al., 2017). The optimal range of different variables in the microwave extraction process was evaluated using screening experiment. Besides, the results in this study showed the prominent antibacterial activity and the antioxidant activity of polysaccharides from *R. laevigata* fruits.

MATERIALS AND METHODS

The fruits of *Rosa laevigata* Michx. (reddish brown) were collected from Guangxi medicinal market in autumn around October, China. The sample (RLSY1609) was stored in our research group laboratory of Shenyang University of Chemical Technology. *Staphylococcus aureus* (*S. aureus*, strain ATCC 25923), *Escherichia coli* (*E. coli*, strain ATCC 25922) and Luria-Bertani (L.B.) medium were procured from Huankai Microbial Sci.&Tech. Co., Ltd. (Guangdong, China). Thiazolyl Blue Tetrazolium Bromide (MTT) was procured from Solarbio Science & Technology Co., Ltd. (Beijing, China). 2, 2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) and 1, 1-Diphenyl-2-picrylhydrazyl radical 2, 2-Diphenyl-1-(2, 4, 6-trinitrophenyl) hydrazyl (DPPH) were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China); D-glucose was obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Petroleum ether, ethanol, and phenol were procured from Shanghai Hengxing Chemical Reagent Co. (Shanghai, China). All the chemicals were of analytical grade.

UV-4100 visible spectrophotometer was purchased from Xinyi Microwave Chemical Technology Co., Ltd., (Shanghai, China). WP800TL23-K3 microwave oven was obtained from Foshan shunde granger microwave electric appliance Co. Ltd. (Foshan, China). RE-52 A rotary evaporator was supplied by BUCHI Labortechnik AG (Shanghai, China).

Extraction of polysaccharides

The dried fruits of *R. laevigata* were ground and sieved to obtain a fine powder. Esters were removed by heating 200 g of the sample in 600 mL of petroleum ether for 8 h at 62 °C. The residue was then refluxed with 80 % ethanol solution (800 mL, 84 °C, 4 h) to remove oligosaccharides and colored materials. Subsequently, the supernatant was discarded, and the residue was dried at 60 °C until a constant weight was obtained and then stored in a desiccator. Further, distilled water was used to extract polysaccharides from the residue using the microwave-assisted extraction method. A 1.0 g sample of the residue in distilled water was incubated in a microwave bath under the pre-determined parameters of microwave power (300-700 W), extraction time (5-25 min), the number of extractions (one-five) and solid-liquid ratio (40-80 mL/g). The polysaccharides were obtained by filtration and enrichment of the extract.

Standard curve of polysaccharides

A D-glucose stock solution of 0.1 mg/mL was used as a standard in this study. D-glucose stock solution (0.2, 0.4, 0.6, 0.8, 1.0 mL) was put in a volumetric flask, and the volume was topped up with distilled water up to 2.0 mL. Subsequently, 5 % phenol (1 mL) and concentrated sulfuric acid (5 mL) were added to each flask, the components were mixed by shaking, and the mixture was maintained in the flask for 30 min at room temperature. A blank solution was prepared using distilled water instead of the D-glucose standard solution. The absorbance of each mixture was determined at a wavelength of 490 nm, and the polysaccharide standard curve was obtained. The concentrations of polysaccharides in the *R. laevigata* fruits extract were determined using this standard curve. The polysaccharide yields were expressed as:

$$\text{Yield} = \frac{C \times V \times N}{W \times 1000} \times 100\% \quad (1)$$

Where C is the polysaccharide concentration (mg/mL), V is the volume of the extract (mL), N is the dilution factor, W is the weight of the *R. laevigata* fruit powder sample (g).

BBD optimization of the extraction

The conditions for microwave-assisted extraction of polysaccharides from *R. laevigata* fruits were optimized using the response surface methodology (RSM). Based on results from the single factor experiment, a three-factor three-level Box-Behnken design (BBD) was designed to examine the microwave-assisted extraction conditions. The conditions explored in this study include microwave power, extraction time, number of extractions, and the liquid-to-solid ratio. The three levels of independent variables (A, the microwave power; B, the extraction time; C, the liquid-to-solid ratio) were expressed in coded values as−1, 0, and 1 respectively. The BBD of three-factor/three-level was designed consisting of 17 experimental points. The independent variables of the experimental design and the experimental data are shown in Tables S1 and S2 respectively. The following second-order polynomial model equation predicted the relationships between the three independent variables:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_i x_i^2 + \sum \beta_{ij} x_i x_j \quad (2)$$

Where Y represents the predicted response, β_0 indicates the intercept regression coefficient, β_i is the linear regression coefficient, β_j illustrates the quadratic regression coefficient and β_{ij} represent the cross-coefficients. X_i and X_j are the independent variables.

Antibacterial activity assay by MTT method

The antibacterial activities were evaluated using the MTT method as described by Oh and Hong (2021) with some modifications. Firstly, dilute *S. aureus* and *E. coli* bacterial solution with LB liquid medium to 10^6 CFU/mL respectively. Polysaccharides samples were dissolved in the diluted bacterial solution to prepare different concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125 mg/mL), a mixture of 100 μL of diluted bacterial solution and 100 μL of samples with various concentrations were seeded into 96 well plates to incubate with 37 °C for 18 hours. Then 15 μL of MTT (5 mg/mL in PBS) was added to each well, and DMSO (100 μL/well) was added after 4 hours of incubation. After 5 minutes, the absorbance was measured at 570 nm wavelength using the enzyme marker detector. In addition, a mixture of 100 μL of LB liquid medium and sample solution (100 μL) were used as a blank, and LB liquid medium (100 μL) and bacterial solution (100 μL) were applied as the control group. The bacteriostatic rate of polysaccharides was calculated according to the following equation:

$$\text{Scavenging rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (3)$$

Where A_0 is the control group (LB liquid medium and bacterial solution); A_1 is the sample group (samples and bacterial solution); A_2 is the blank group (samples and LB liquid medium).

Antioxidant activity assay

a) DPPH radical scavenging assay

The DPPH radical scavenging assay was evaluated based on previous literature reports (Giuffrè et al, 2017; Sharma and Kumar 2011) with some modifications. Briefly, polysaccharide samples were dissolved in 50 % ethanol to prepare different concentrations (0.005, 0.020, 0.080, 0.300, 0.800, 2.000, 5.000 mg/mL), a mixture of 100 μL of DPPH ethanol solution (0.2 mmol/L) and 100 μL of samples with various concentrations were seeded into 96 well plates. The mixture was incubated for 30 min in dark at room temperature. Then, the DPPH radical scavenging activity was gauged at 517 nm using multimode reader (Bertholdd LB943). In addition, a mixture of 100 μL of DPPH ethanol solution (0.2 mmol/L) and 50 % ethanol (100 μL) were used as a blank, and Vitamin C instead of polysaccharide as a positive standard. The DPPH radical scavenging rate was calculated according to the following equation:

$$\text{Scavenging rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (4)$$

Where A_0 is the control group (DPPH solution and 50 % ethanol solution); A_1 is the sample group (samples and DPPH solution); A_2 is the blank group (samples and 50 % ethanol solution).

b) ABTS radical scavenging assay

The ABTS radical scavenging activity was evaluated based on previous literature reports (George et al, 2022; Giuffrè et al, 2018) with minor modification. Briefly, the aqueous solutions of ABTS (7 mmol/L) and $K_2S_2O_8$ (2.45 mmol/L) were mixed in equal volume and reacted for 12-16 hours in the dark at room temperature. Then, the mix solution of ABTS and $K_2S_2O_8$ was diluted with absolute ethanol to make its absorbance range within 0.70 and 0.02 at 734 nm. After that, ABTS mixed solution (190 μL) and various concentrations (0.005, 0.020, 0.080, 0.300, 0.800, 2.000, 5.000 mg/mL) of 50 % ethanol sample solution (10 μL) were seeded to a 96-well plate. The mixture was incubated for 10 min in the dark at room temperature, then the ABTS radical scavenging activity was gauged at 734 nm using multimode reader (Bertholdd LB943). In addition, 190 μL of ABTS mixed solution and 50 % ethanol (10 μL) were used as a blank, and Vitamin C instead of polysaccharide as a positive standard. The ABTS radical scavenging rate was calculated according to the following equation:

$$\text{Scavenging rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (5)$$

Where A_0 is the control group (ABTS solution and 50 % ethanol solution); A_1 is the sample group (samples and ABTS solution); A_2 is the blank group (samples and 50 % ethanol solution).

RESULTS AND DISCUSSION

Compared with traditional extraction methods, microwave-assisted extraction has the advantages of significantly reducing the extraction time, consuming less solvent and effectively increasing the extraction rate. (Bhan et al., 2017; Gabriel et al., 1998; Zhao et al., 2016). This study optimized the process of microwave assisted extraction of polysaccharides from *R. laevigata* fruit (Azahar et al., 2017) and selected the optimal range of different variables during the microwave extraction process.

Screening experiment

a) Effects of the microwave power

The effect of microwave power on the extraction efficiency was determined by performing the tests at microwave power ranging from 300 to 700 W. Meanwhile; other extraction conditions were fixed as follows: extraction time (15 min), the number of extractions (two), liquid-to-solid ratio (40 mL/g). As indicated in Fig. 1a, an increase in microwave power from 300 to 500 W increased polysaccharide yield. However, the extraction yield decreased markedly when the microwave power was increased from 500 to 700 W. The observed trend could suggest that a microwave power beyond 500 W damaged the structure of polysaccharides (Kölln et al., 2016; Maran et al., 2013; Wang, Y.H. et al., 2014). An optimal extraction power of 400-600 W was chosen.

b) Effects of the microwave time

The impact of the extraction time (5-25 min) polysaccharide yield is illustrated in Fig. 1b. These experiments included two extractions done at a microwave power of 500 W and a liquid-to-solid ratio of 40 mL/g. An increase in extraction time from 5 to 20 min increased polysaccharide yield. Further extension of extraction time from 20 to 25 min resulted in a decrease in polysaccharide yield. Consequently, an extraction time of 15-25 min was adopted as the optimal.

c) Effect of the number of extractions

The impact of the number of extractions on the polysaccharide yield is presented in Fig. 1c. The experiments were performed a microwave power of 500 W, an extraction time of 15 min, and a liquid-to-solid ratio of 40 mL/g. Increasing the number of extractions from one to three increased polysaccharide yield. However, an increase beyond three extractions did not significantly affect the polysaccharide yield. Thus, two extractions were determined as optimal to save on cost and time.

d) Effects of the liquid-to-solid ratio

The impact of the liquid-to-solid ratio (40-80 mL/g) on polysaccharide yield was evaluated by doing two extractions at a microwave power of 500 W for 15 min extraction time. The results are as shown in Fig. 1d. It was found that polysaccharide yields increased as the liquid-to-solid

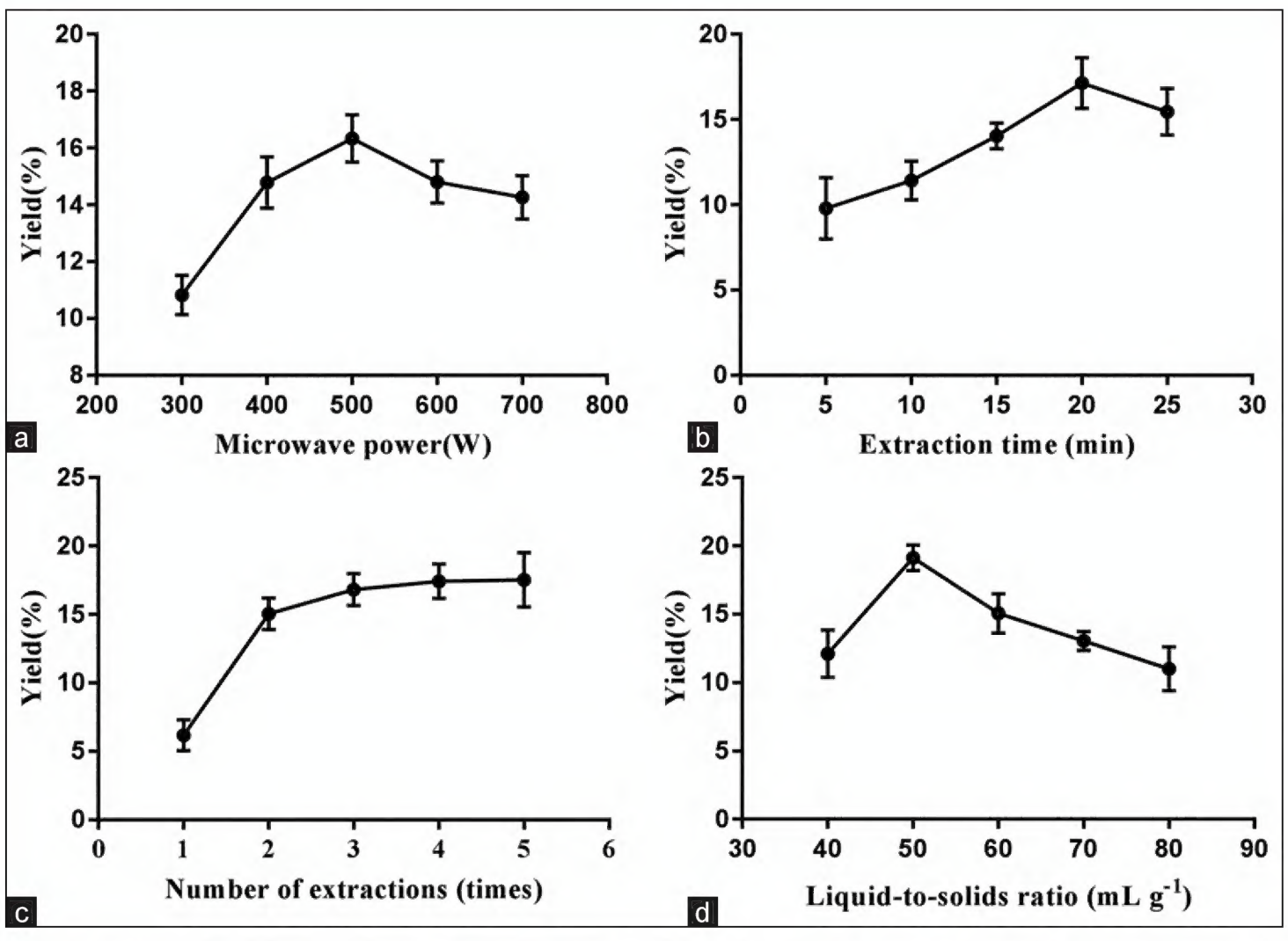

Fig 1. Effects of microwave power (a), extraction time (b), number of extractions (c), liquid-to-solids ratio (d) on extraction yield of polysaccharides

ratio increased from 40 to 50 mL/g The yields, however, declined significantly when the liquid-to-solid ratio exceeded 50 mL/g. The decline could be attributed to an excess of solvent dissipating cavitation energy from the extraction system and negatively impacting the extraction procedure (He et al., 2014; Zhang,Bai, 2011). A liquid-to-solid ratio of 40-60 mL/g was, therefore, regarded as the optimum for a Box-Behnken design (BBD) experiment.

Optimization of microwave-assisted extraction conditions of polysacc harides by RSM

The effect of the optimized conditions on the response variables was determined using Design Expert 8. Experimental data were fitted to a quadratic polynomial model as shown in the following equation:

$$Y\% = 20.32 + 0.62A - 0.23B + 0.36C + 0.064AB + 0.47AC - 0.29BC - 2.36A^2 - 0.39B^2 - 0.66C^2 \quad (6)$$

Where *A* is the microwave power (W), *B* is the extraction time (min), and *C* is the liquid-to-solid ratio (mL/g).

Analysis of variance (ANOVA) was done to evaluate the predictive model, and the results are as presented in Table 1. The results suggest that the model fitness was highly significant when the probability value was low ($p < 0.001$). The coefficient R^2 and the adjusted R^2_{adj} were 0.9685 and 0.9281, respectively, which implied that the regression model represented the experimental data well. Also, the predicted R^2_{pred} was 0.8161, showing the predicted model was excellent. Moreover, the lack of fit was significant. As a result, the fitness model of the response surface methodology (RSM) was effective.

The relevance of each of the factors was illustrated using a 3D response surface and 2D contour plots. The effects of the microwave power and the extraction time are as indicated in Figs. 2a and 3a respectively. The highest polysaccharide yield was obtained when the microwave power and the extraction time were at medium levels (0 levels). When the microwave power was maintained constant, the yields initially increased from the 15 to the 19 min but decreased beyond the 19 min. At a fixed microwave power, the yield initially increased, after which it decreased with an increase in extraction time. Figs. 2b and 3b indicate that the interaction between the microwave power and the liquid-to-solid ratio was significant. Higher yields were obtained when the microwave power and the liquid-to-solid ratio were kept at medium levels (0 levels). It could, therefore, be concluded that polysaccharide yields increased rapidly following an increase in the liquid-to-solid ratio (*C*) from 40 to 51 mL/g, and a microwave power (*A*) increase ranging from 400 to 480 W. Figs. 2c and 3c demonstrate that the relationships between the extraction time and liquid-to-solid ratio was clearly affected the yield.

Optimization experimental verification

The predictive model was validated under the optimal factors: *A* of 516 W; *B* of 18 min; *C* of 54 mL/g. As shown in Table S3, the average yield (20.37 %) of the optimal experiment was comparable to the average value (20.40 %) of the predictive model. Hence, the microwave-assisted extraction conditions of polysaccharides from *R. laevigata* fruits optimized by RSM were accurate and feasible.

Antibacterial activity test analysis

The minimum inhibitory concentration (MIC) is essential for assessing antimicrobial activity (Wang, B. et al., 2014; Zhang et al., 2005). As presented in Table S4, the samples as gradient concentration ranged from 0.78-100 mg/mL. It could be demonstrated from Fig. 4 that there is an obviously positive correlation between the anti-bacterial activities and concentrations of samples. The inhibition of

Table 1: Analysis of variance for the response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	P-value Prob>F	Significance
Model	33.20	9	3.69	23.95	0.0002	***
A	3.08	1	3.08	19.96	0.0020	**
B	0.42	1	0.42	2.75	0.1410	
C	1.03	1	1.03	0.70	0.0360	*
AB	0.016	1	0.016	0.11	0.7539	
AC	0.80	1	0.80	5.75	0.0476	*
BC	0.35	1	0.35	2.26	0.1765	
A^2	23.50	1	23.50	152.55	0.0001	***
B^2	0.63	1	0.63	4.09	0.0828	
C^2	1.85	1	1.85	12.01	0.0105	*
Residual	1.08	7	0.15			
Lack of Fit	0.32	3	0.11	0.56	0.6680	
Pure Error	0.76	4	0.10			
Cor Total	34.28	16				

[a]*p<0.05, **p<0.01 and ***p<0.001; [b]the microwave power (*A*), the extraction time (*B*), and the liquid-to-solid ratio (*C*).

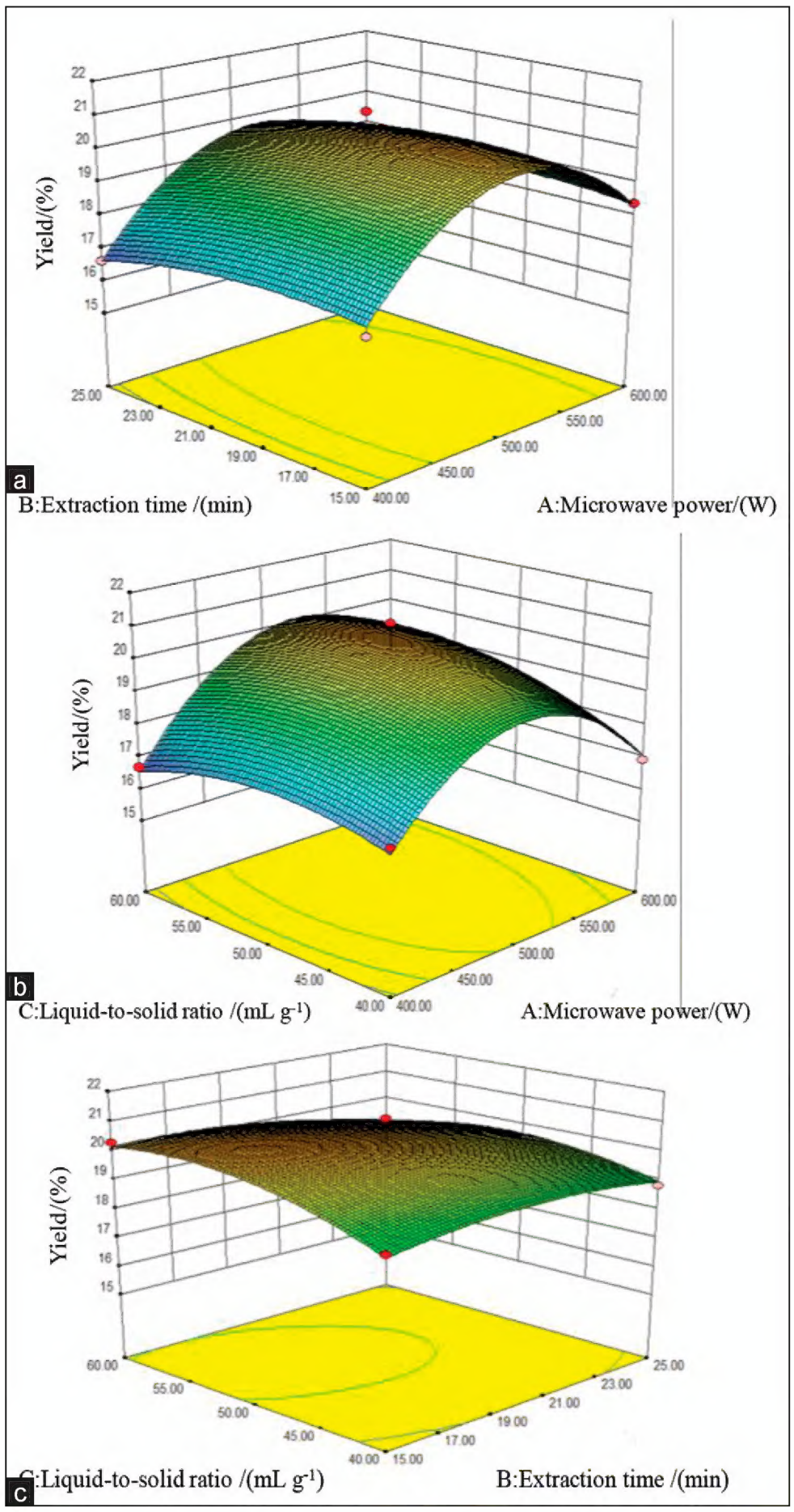

Fig 2. Three-dimensional response surfaces graphs for interaction between microwave power and extraction time (a), microwave power and liquid-to-solids (b), extraction time and liquid-to-solids (c).

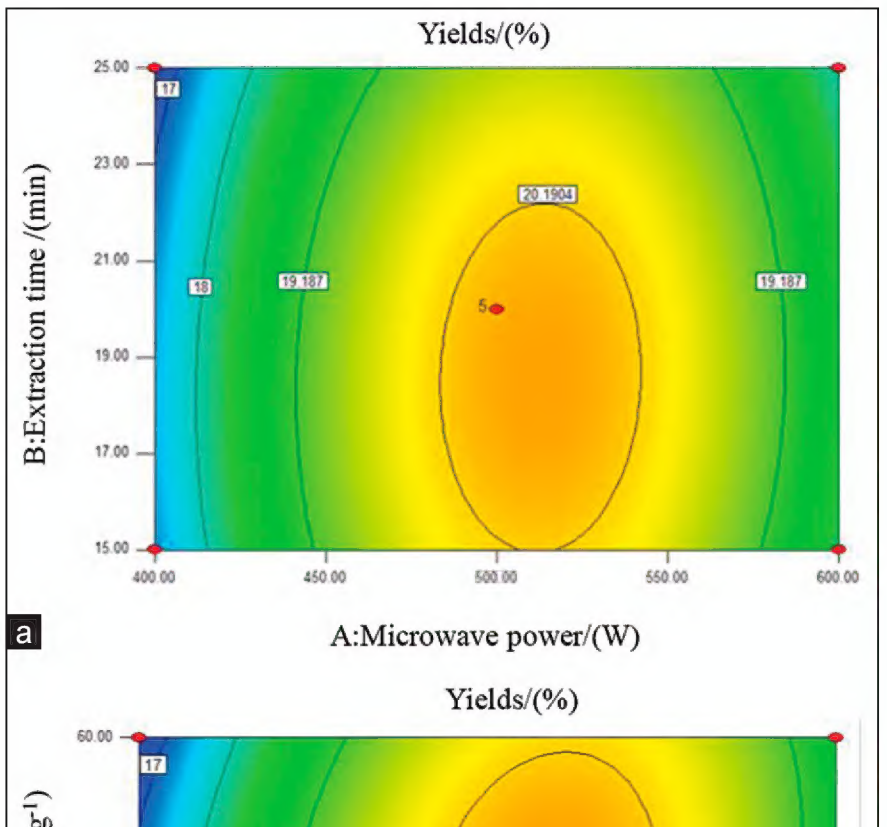

Fig 3. The contour plots for interaction between microwave power and extraction time (a), microwave power and liquid-to-solids (b), extraction time and liquid-to-solids (c).

polysacchrides on *E.coli* is better than *S.aureus* at various concentrations. At the concentration of 100mg/mL, inhibition radio of *E.coli* and *S.aureus* could be reached at 74.66 % and 67.18 %, respectively. Furthermore, the MIC values of polysaccharides are 1.56 mg/mL and 3.13 mg/mL against the *E.coli* and *S.aureus*, respectively. The results of this study indicate that polysaccharides from *R. laevigata* fruits possess excellent antibacterial activities.

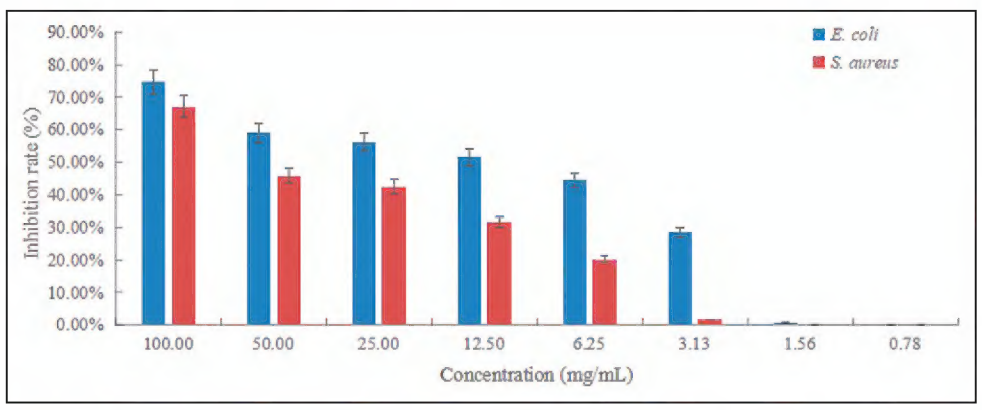

Fig 4. Anti-bacteria activity of polysaccharides of *R.laevigata* against *E. coli* and *S. aureus*.

Antioxidant activity test analysis

a) DPPH radical scavenging activity

Because antioxidants can scavenge DPPH free radicals, DPPH method is a common method to study the

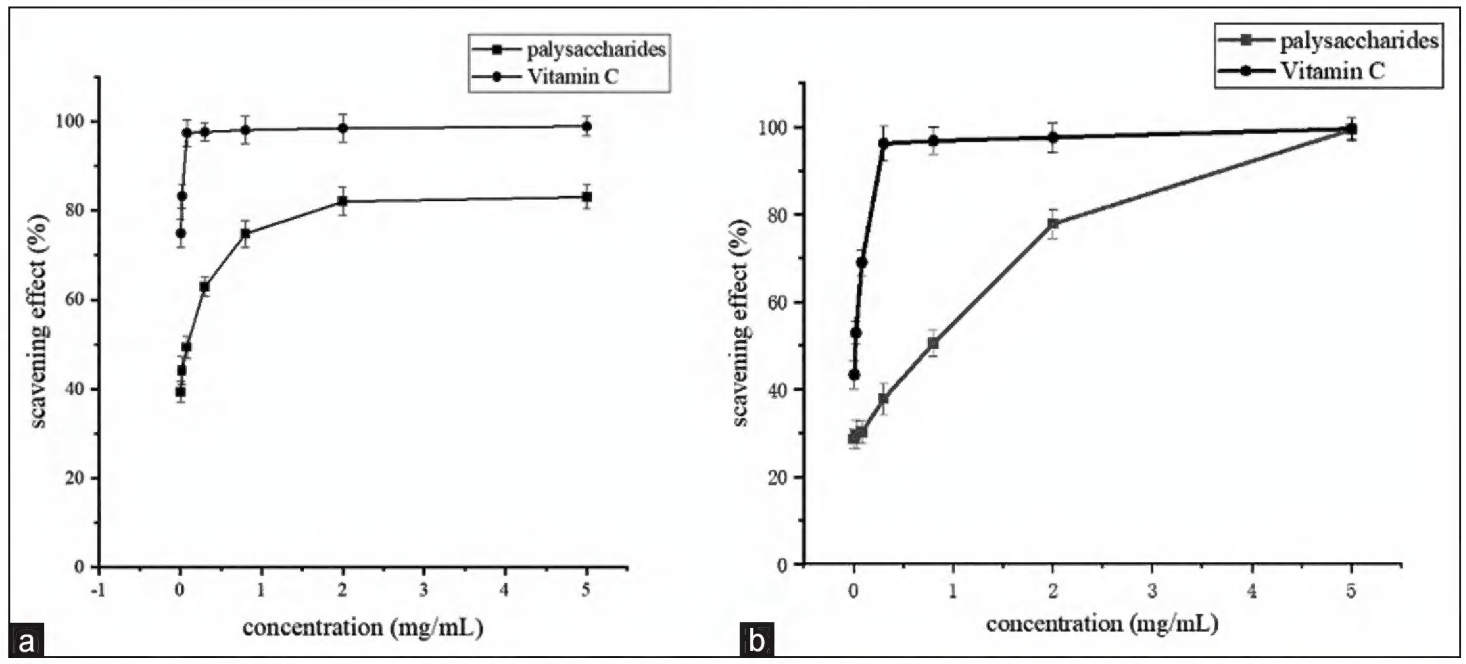

Fig 5. Scavenging activities of polysaccharides and Vitamin C on DPPH radicals (a) and ABTS radicals (b). Data are expressed as the mean ± SD, n = 3.

Table 2: Comparison of microwave-assisted extraction with reflux extraction in extraction conditions and yields.

Extraction methods	Extraction time	Number of extraction	Liquid-to-solid ratio (mL/g)	Microwave power (W)/ Temperature (°C)	Yield
Optimized microwaveassisted extraction	18 min	3	54	516 W	20.37%
Reflux extraction	1 h	3	54	90°C	17.58%

antioxidant activity of natural compounds *in vitro*. The DPPH radical scavenging activities of the polysaccharides and Vitamin C at various concentrations are shown in Fig. 5a. The results showed that polysaccharides had a dose-dependent scavenging ability of DPPH free radicals, the scavenging effects were 83.06 % at concentration of 5.0 mg/mL. At the same concentration, the scavenging effects of Vitamin C were 98.92 %.

b) ABTS radical scavenging activity

The ABTS radical scavenging activities of the polysaccharides and Vitamin C at various concentrations are shown in Fig. 5b. The results showed that the scavenging effects of polysaccharides were 99.38 % at concentration of 5.0 mg/mL, as good as Vitamin C. The scavenging effects of Vitamin C were 99.6 % at the same concentration. So, the polysaccharides can remarkably scavenge ABTS radicals, especially at high concentrations.

In conclusion, the polysaccharides of *R. laevigata* have good antioxidant activity *in vitro*.

Comparison of polysaccharide yields under optimized microwave-assisted extraction conditions and by reflux extraction

The reflux extraction fixed as follows: extraction time of 1 h, number of extractions three, liquid-to-solid ratio of 54 mL/g and temperature of reflux of 90 °C. The average yields of the optimized microwave extraction process and the traditional reflux extraction of the water bath were 20.37 % and 17.58 %, respectively. The results showed that the polysaccharide yield of microwave-assisted extraction under optimized conditions were higher than the reflux extraction method (Table 2). Therefore, the microwave extraction assisted process has good stability, high efficiency and certain practical application value.

CONCLUSIONS

As a traditional Chinese medicinal herb with wide distribution, *R. laevigata* has rich efficacy and wide clinical application, mainly focusing on antioxidant, anti-inflammatory and antibacterial. A variety of medicines made from *R. laevigata*, such as Fukeqianjin tablets, Sanjin tablets and Jinji capsules, have remarkable clinical efficacy. However, the proven biological functions are mainly focused on extracts, such as total polysaccharides, total flavonoids. In this study, the response surface methodology (RSM) was used to optimize the microwave-assisted extraction conditions for polysaccharides of *R. laevigata* fruits on the single-parameter experiments. The results of the verification experiment showed that the regression model was adequate and reliable. Besides, previous studies have also demonstrated that RSM does not only reduce the number of experiments but can also be used to evaluate and optimize complex processes compared to traditional methods effectively. This study, therefore, widely used RSM to optimize the polysaccharides extraction process. Optimal extraction conditions were found to be a microwave power of 516 W, an extraction time of 18 min, and a solid-liquid ratio of 54 mL/g. The optimal conditions gave a maximum polysaccharides yield of 20.37 %. Although microbial infections have plagued humans for many years,

there has been little research focussed on the antibacterial activity of polysaccharides from *R. laevigata* fruits. The results of this study indicate that the polysaccharidess of *R. laevigata* fruits have functional antibacterial activities and have excellent antioxidant activity *in vitro*. The MIC of polysaccharides against *E.coli* and *S.aureus* were 1.56 mg/mL and 3.13 mg/mL, respectively. Scavenging activities of polysaccharides on DPPH and ABTS could be reached at 83.06 % and 99.38 %, respectively. Studies have shown that polysaccharides with proven antibacterial, antioxidant and biocompatible functions are often used to preserve foods or improve their physicochemical properties (Zhang et al., 2022). The excellent antimicrobial and antioxidant activities exhibited by *R. laevigata* polysaccharide make it possible to be developed as a food preservative. Finally, *R. laevigata* fruits have a wide range of research prospects and medicinal value, and further studies should be carried out to explore other biological activities of *R. laevigata* fruits, which would offer significant theoretical and practical importance.

SUPPLEMENTARY INFORMATION

When applicable, use this section to inform the reader that "Supplementary Information (detail here the kind of information) is available free of charge at Supplementary Information.docx.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Dr. Pinyi Gao is responsible for directing the project and for writing this article; Xin Shen and Yongdan Guo carried out the experiment; Dandan Yue and Mei Jina carried out the data and figures; Dr. Changfeng Liu served as advisors for the writing of the article and the interpretation of the data; Dr. Danqi Li and Dr. Xuegui Liu headed the experimental work of the project in the laboratory.

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LIST OF SUPPLEMENTARY CONTENT

Table S1: Coded and actual values in the Box-Behnken Design for the extraction of polysaccharides

Variables	Factors	Code		
		Lower (–1)	Central point (0)	High (+ 1)
A	Microwave power (W)	400	500	600
B	extraction time (min)	15	20	25
C	liquid-to-solid ratio (g/mL)	40:1	50:1	60:1

Table S2: Box-Behnken design and experimental results of polysaccharide yields

Runs	A	B	C	Polysaccharide yields (%)
1	0	0	0	20.171
2	0	0	0	20.108
3	1	0	1	18.567
4	0	1	1	19.005
5	0	0	0	20.113
6	0	0	0	20.116
7	0	0	0	21.099
8	1	1	0	18.304
9	1	0	–1	16.943
10	0	–1	–1	18.947
11	0	1	–1	18.842
12	–1	–1	0	16.968
13	–1	0	–1	16.966
14	1	–1	0	18.402
15	–1	1	0	16.614
16	0	–1	1	20.291
17	–1	0	1	16.708

Table S3: Comparison of predicted and experimental values

Runs	Predictive value (%)	Experimental value (%)	Relative error(%)
1	20.43	20.40	0.15
2	20.31	20.26	0.25
3	20.46	20.45	0.05
Average value	20.40	20.37	0.15

Table S4: The Inhibition rate of *S. aureus* and *E. coli* at different polysaccharide concentrations from *R. laevigata* fruits

Polysaccharide concentration	Inhibition rate of *S. aureus*	Inhibition rate of *E. coli*
0.78 mg/mL	0.00%	0.00%
1.56 mg/mL	0.00%	0.69%*
3.13 mg/mL	1.67%*	28.49%
6.25 mg/mL	20.21%	44.59%
12.50 mg/mL	31.53%	51.69%
25.00 mg/mL	42.60%	56.08%
50.00 mg/mL	45.95%	59.12%
100.00 mg/mL	67.18%	74.66%

*the MIC